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SCIENCE OF LIGHT

VOLUME 7 NUMBER 1

March

1958



Published by the

Institute for Optical Research

Tokyo University of Education

in collaboration with

The Spectroscopical Society of Japan

SCIENCE OF LIGHT

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Printed at

the Printing Department, Chūō Kagakusha,
Tokyo

Achromatic Anti-reflection Coating for Practical Use

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(Received February 26, 1958)

Abstract

Non-absorbing film is made by vacuum-evaporating Sb_2O_3 using alumina heater. Achromatic anti-reflection coating is made by combining this film with MgF_2 film. The coating is sufficiently durable for practical use.

1. Introduction

Theory of achromatic anti-reflection coating has been known since about ten years ago^{1,2)}. The difficulty in obtaining non-absorbing and durable thin films of desired indices, however, prevented achromatic coating to be made for practical use. The author obtained films, non-absorbing for visible light and durable by vacuum evaporation of antimony oxide (Sb_2O_3), and worked out the achromatism of the system MgF_2 ($\lambda_0/4$)- Sb_2O_3 ($\lambda_0/2$)-glass where λ_0 represents the central wavelength of the coating and studied the fundamental problems for industrializing the production which has become promising by co-operation of interested workers.

2. Theoretical consideration of the achromatic coating

In a schematic diagram of the coating, Fig. 1, n 's and d 's represent the refractive indices and the geometrical thicknesses. Assume that all the media are non-absorbing and the incident light is normal to the surface of the film system. When for the phase angle $\delta_i = (2\pi/\lambda)n_i d_i$ ($i=1, 2$)

$$\delta = \delta_1 = (1/2)\delta_2, \quad (1)$$

and the condition between the refractive indices

$$n_2 n_0 (n_1 + n_2) (n_0^2 + n_1^2) = 2 n_0 n_1 (n_2^2 + n_1 n_2^2) \quad (2)$$

is satisfied, the reflected light from the coating is extinguished at the phase angle δ which is given from the relation

$$\cos^2 \delta = n_2 (n_0 n_0 - n_1^2) / 2(n_1 + n_2)(n_0 n_0 - n_1 n_2). \quad (3)$$

Putting $n_0 = 1$ (for air), solutions for the achromatism of coating on various glasses (n_0) are given in Fig. 2 graphically, where ϵ represents the angular distance of zero reflectance points of the coating in terms of the phase angle.

1) A. F. Turner, *J. Physique et Radium* **11**, (1950) 444.

2) A. Vasicek, *Optica Acta*, May, 1951, 20.

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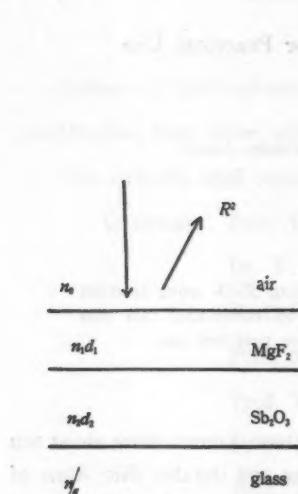


Fig. 1. Schematic diagram of the achromatic coating.

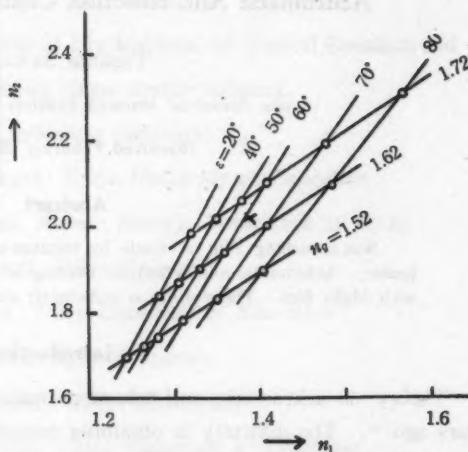


Fig. 2. Graphical representation of solutions for the achromatism of coating, where ϵ represents the angular distance between the reflection minima.

As shown later, evaporated film of Sb_2O_3 has the refractive index of 2.02 in the neighbourhood of the centre of visible region. Thus, a double layer system consisting of MgF_2 $\lambda_0/4$ layer ($n=1.39$) and Sb_2O_3 $\lambda_0/2$ layer becomes an achromatic coating for glass $n_g=1.665$, and in this case $\epsilon=61^\circ$ (the point \times in Fig. 2).

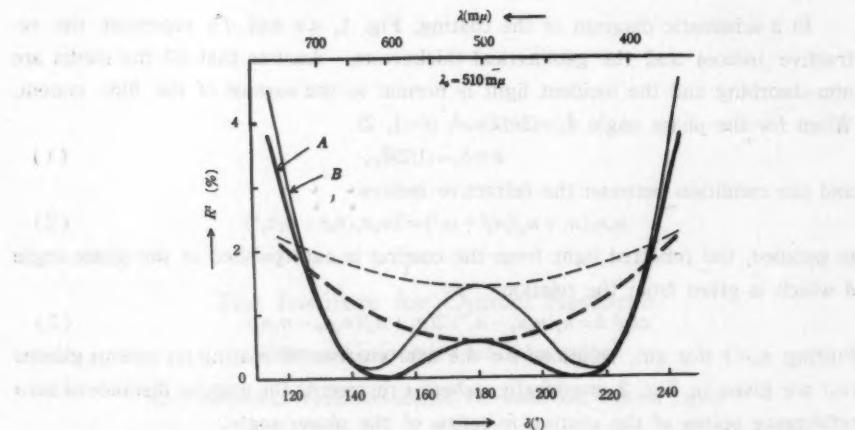


Fig. 3. Reflectance of the coating. A is for the achromatic coating on glass $n=1.665$, B on glass $n=1.52$, and dotted lines are the respective MgF_2 monolayer coatings on the glasses.

The theoretical spectral reflectance of the coating is shown as the curve *A* in Fig. 3, where dotted thick line shows the reflectance of MgF_2 mono-layer coating.

When the above double layer coating is used on various glasses, the reflectance minima R^2_{\min} , the angular distance ε and the central maximum R^2_{\max} vary as shown in Fig. 4.

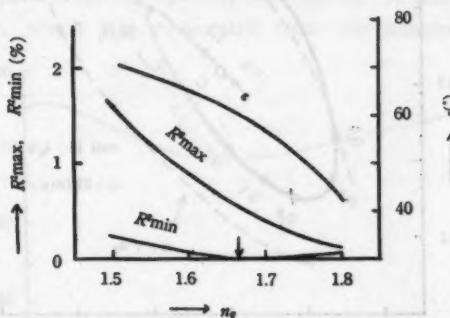


Fig. 4. R^2_{\min} , R^2_{\max} and ε of reflectance curves of the achromatic coating ($n_1=1.39$, $n_2=2.02$) on various glasses (n_g).

Thus, the coating is efficiently achromatic for glasses $n_g=1.5 \sim 1.75$. For example, theoretical spectral reflectance of the coating for glass $n_g=1.52$ is shown as curve *B* in Fig. 3.

Next, let us consider the interference color of the light reflected from the film system for the control of the coating process. With the central wave-length of the coating $\lambda_0=510 \text{ m}\mu$ and $n_g=1.52$, the interference color for the *C* light-source during the coating varies on C. I. E. diagram as shown in Fig. 5. In the figure, the dotted line represents the interference color of an ideal mono-layer of $n=\sqrt{n_g}$ ³⁾, and the point \odot that of the achromatic coating on glass $n_g=1.665$ (shown as the curve *A* in Fig. 3). It can be seen from the figure that, first, the thickness control of $Sb_2O_3 \lambda_0/2$ film is not difficult, for it is done in the neighbourhood of the sensitive color region³⁾ of this film. Second, the control of $MgF_2 \lambda_0/4$ film is also not difficult, for the interference color corresponding to nearly desired thickness of the film is of excellent purity and is fairly sensitive to the variation of film thickness.

Effects of the variations of n_1 and n_2 and those of the variation of n_g (Fig. 4) on R^2_{\min} , R^2_{\max} and ε are of the same order. Effect of errors in the estimation of film thickness on the spectral reflectance of the coating can be ignored. Hence, no appreciable effect of the incident angle of light is existent for the spectral

3) H. Kubota, J. O. S. A. **40**, (1950) 146.

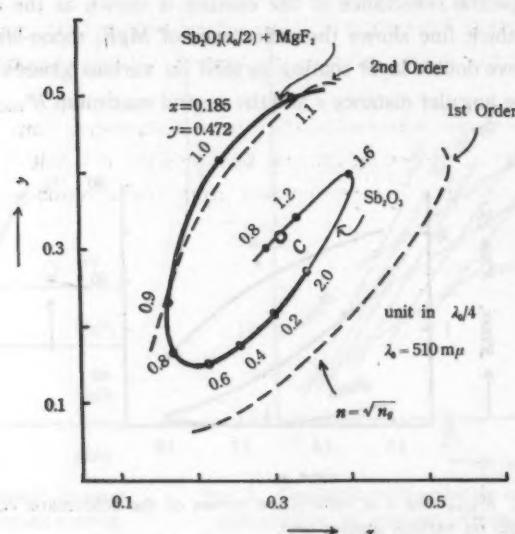


Fig. 5. Variation of interference color of the achromatic coating during the coating on glass $n_g=1.52$ and ideal mono-layer for reference.

reflectance of the coating⁴⁾. For example, when the incident angle of light varies from 0° to 20° , only the positions of minimum and maximum values of the spectral reflectance are displaced toward shorter wave-length side by several percent of the wave-lengths.

3. Vacuum evaporation of Sb_2O_3

If Sb_2O_3 is evaporated in vacuum from a conventional metal heater, the substance sublimes and a film is condensed. But, the film thus deposited is considerably absorbing in shorter wave-length region of visible light. This absorption seems to be due to the decomposition of original substance, for a large increase of the pressure is observed during the evaporation. However, the author and his co-workers found that the use of a conventional metal heater covered with alumina (called the alumina-heater hereafter) suppressed the decomposition, and in consequence, the evaporated film is practically non-absorbing for visible light. And, the film thus evaporated showed the sharp rings of Sb_2O_3 (cubic crystal) in electron diffraction study⁵⁾.

In spite of the use of alumina-heater, a slightly absorbing film is condensed

4) T. Sawaki and S. Tabata, Bull. Osaka Ind. Research Inst. 5, (1954) 81.

5) T. Sawaki, M. Iwata, Y. Watanabe, T. Fukuda and S. Tabata, ib. 5, (1954) 211.

at higher evaporating temperatures. The proper temperature should be about or below 1100°C at which the increase in pressure during evaporation was found slight.

Spectral reflectances and transmittances of evaporated films were measured with a G. E. automatic-recording spectral photometer. A result of measurement with a Sb_2O_3 film, which was evaporated from the alumina-heater below the

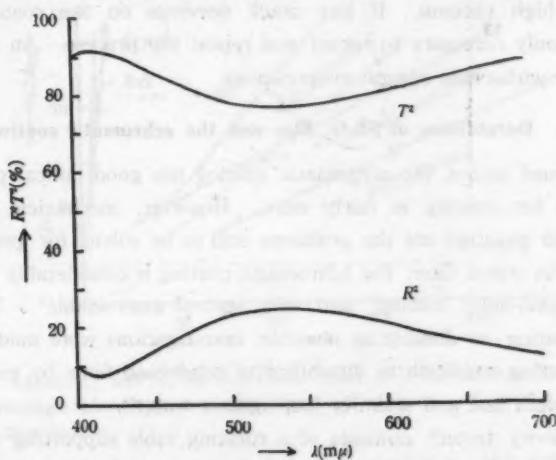


Fig. 6. Observed spectral reflectance and transmittance of Sb_2O_3 film.

pressure 1×10^{-4} mmHg and condensed on glass at a room temperature, is shown in Fig. 6. Refractive index of the film calculated from the curve in the figure is 2.02.

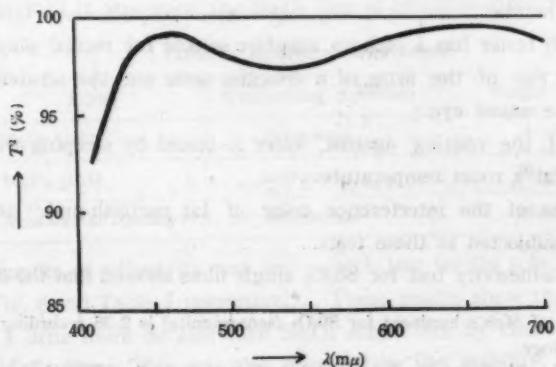


Fig. 7. Observed spectral transmittance of a glass plate with the achromatic coating on both surfaces.

The achromatic coating can be prepared with Sb_2O_3 film thus obtained and a conventional MgF_2 film. Observed spectral transmittance of a glass plate of BK 7 with the achromatic coating on both surfaces is shown in Fig. 7.

Aside from the way of producing the alumina-heater already reported⁷⁾, the following procedure was adopted: alumina powder is kneaded with very dilute solution of water glass and applied on a conventional metal heater, which is then air-dried and baked in high vacuum. If any crack develops on the coated alumina in baking, it is only necessary to recoat and repeat the process. An alumina-heater thus produced withstands several evaporation.

4. Durabilities of Sb_2O_3 film and the achromatic coating

As mentioned before, the achromatic coating has good optical properties, and the procedure for coating is fairly easy. However, mechanical durability and stability against moisture are the problems still to be solved for practical use of the coating. As stated later, the achromatic coating is considerably frail compared with MgF_2 mono-layer coating, and this seemed unavoidable*. To obtain the achromatic coating as durable as possible, investigations were made on the relation of evaporating condition to durability of condensed films by means of adhesivity test, scratch test and stability test against water⁶⁾.

The adhesivity tester⁸⁾ consists of a rotating table supporting a coated glass sample and a bundle of glass fibres, the latter is pushed normally to the film surface with a given weight (W gr) and moved to and fro. The rotation of the table is mechanically coupled to the motion of the fibre bundle. The adhesivity is represented in terms of the number of revolution of the table with which number the rubbed-off area by the fibre bundle becomes the same as that of the section of the bundle.

The scratch tester has a pick-up sapphire needle for record player (S.P.) set downward on one of the arms of a druggist scale and the scratched marks are observed by the naked eye.

Stability of the coating against water is tested by steeping the sample into distilled water at a room temperature.

Single films of the interference color of 1st purplish-pink⁹⁾ and achromatic coatings were subjected to these tests.

Results of adhesivity test for Sb_2O_3 single films showed that the film condensed

* The value of Moh's hardness for Sb_2O_3 (senarmontite) is 2.35 according to a text book of mineralogy.

6) T. Sawaki, M. Iwata, S. Tabata and S. Katsube, ib. 9, (1958) 11.

7) L. O. Olsen, C. S. Smith and E. C. Crittenden, J. App. Phys. 16, (1945) 425.

8) M. G. Townsley, R. S. I. 16, (1945) 143.

on glass at $100^{\circ}\text{C} \sim 150^{\circ}\text{C}$, and then heated at $250^{\circ}\text{C} \sim 300^{\circ}\text{C}$ for about 20 minutes, seemed most adhesive. At higher condensing temperatures, the time needed for obtaining the required film thickness was longer; the higher the temperature, the steeper becomes the rate of increase of the time. The films lost about 5% in thickness in an after-treatment at 300°C due to re-vaporization which increased steeply in amount as the temperature was raised.

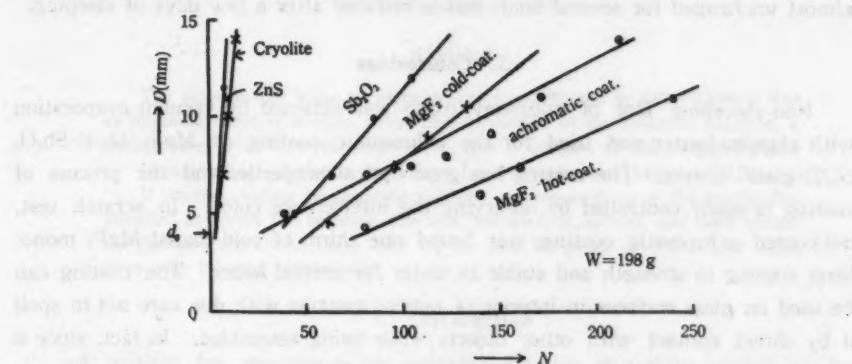


Fig. 8. Adhesivity test for various films, where N is the number of revolution of the rotating table, D the diameter of rubbed-off area and d_0 the diameter of glass fibre bundle.

Some results of the scratch test, supplemented to the adhesivity test, are shown in Table I. It is seen from the table that Sb_2O_3 film is about $1/6 \sim 1/7$ of cold-coated MgF_2 film in strength.

From the above results, the achromatic coating made in the following way seemed most durable: first, $\text{Sb}_2\text{O}_3 \lambda_0/2$ film was coated on glass at $100^{\circ}\text{C} \sim 150^{\circ}\text{C}$, and next, $\text{MgF}_2 \lambda_0/4$ film upon the Sb_2O_3 film at condensing temperature of 250°C .

Table I. Results of scratch test.

Films	Evaporating condition	Weight (gr.)
$\text{Sb}_2\text{O}_3 (\lambda_0/2)$	hot-coated	4~6
$\text{MgF}_2 (\lambda_0/4)$	cold-coated	30~40
Achromatic coating	hot-coated	10~14

Some results of adhesivity test and scratch test for the achromatic coating are shown in Fig. 8 and Table I respectively. These results show that the achromatic coating is a little more durable than Sb_2O_3 single film by virtue of the protecting action of MgF_2 film. This was also observed in the stability test against water described below.

Both the stability of Sb_2O_3 film and that of achromatic coating against water had no regular relations to evaporation conditions. Defects showing abnormal scattering seems to appear on Sb_2O_3 films in a few days of steeping in water and on achromatic coatings, in about a week. Unaffected regions of the films soften from top surfaces, and after about a week, Sb_2O_3 single films are easily rubbed off completely with a cotton cloth. For achromatic coatings, the adhesivity remains almost unchanged for several hours but is reduced after a few days of steeping.

5. Conclusions

Non-absorbing film of antimony oxide was obtained by vacuum evaporation with alumina-heater and used for the achromatic coating of MgF_2 ($\lambda_0/4$)- Sb_2O_3 ($\lambda_0/2$)-glass system. The coating has good optical properties and the process of coating is easily controlled by observing the interference color. In scratch test, hot-coated achromatic coating was found one third of cold-coated MgF_2 monolayer coating in strength and stable in water for several hours. The coating can be used on glass surfaces in interior of optical systems with due care not to spoil it by direct contact with other objects while being assembled. In fact, since a few years ago, the achromatic coating has been in use on some camera lenses and proven to be effective in color photography⁹⁾.

Non-absorbing film of Sb_2O_3 may no doubt be used as an element of interference filters¹⁰⁾.

Acknowledgements

The author wishes to express his sincere appreciation to Dr. M. Iwata for encouragement and advice given throughout the work. He is deeply indebted to Mr. S. Tabata and Mrs. S. Katsume for invaluable co-operation, to Dr. Y. Watanabe and Mr. T. Fukuda for the help in electron diffraction studies and photometric measurements, to Messrs Y. Matsuda and M. Suzuki of Konishiroku Photo Ind. Co. Ltd for assisting the author in part of the study and to Messrs Y. Tajima, H. Mukai and H. Nishino for the effort in industrializing the coating.

9) H. Nishino, J. App. Phys. Japan., to be published.

10) W. L. Barr and F. A. Jenkins, J. O. S. A. **46**, (1956) 141.

Effect of Using Siemens Star as Scanning Chart in Measurement of Response Function

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(Received March 1, 1958)

Abstract

The error in the value of response function obtained by using Siemens star as scanning chart is studied. For the calculation of the response function, a circular area with uniform intensity of light is adopted as the image of point.

Numerical calculation is performed by the electronic computer FUJIC.

If correct values of response function are required up to the spatial frequencies at which the response function has the first, second, and third zeroes, the Siemens stars with more than 16, 24, and 32 pairs of white and black lines may be used respectively.

1. Introduction

A method for measuring the response function of optical systems has been developed, in which a line image or point image formed by the optical system under test is scanned by a chart with transmission varying sinusoidally¹⁾. Instead of the chart with sinusoidal transmission, the chart consisting of black and white parallel lines, so-called "Foucault chart" can be used and values obtained with it are reduced to the corresponding values which would be obtained with the sinusoidal chart²⁾.

Lindberg proposed an ingeneous method of measuring the response function³⁾. Rotating the Siemens star in the plane of a point image, the light transmitted through the chart is received by a photo-tube and only the fundamental frequency component in the output current of the photo-tube is amplified. The value of the response function measured by this method seems to give without reduction the correct value of response function.

This method has an advantage in that the spatial frequency is easily changed continuously by displacing the point image in radial direction of the star. But on the other hand, as the spatial frequency changes along the radius vector of the star, it may give values different from those measured by using Foucault chart with black and white lines arranged parallel one another and in equal distances.

1) P. Lindberg: *Optica Acta* **1** (1954) 80.

2) J. W. Coltman: *J. Opt. Soc. Am.* **44** (1954) 468.

3) P. Lindberg: *loc. cit.*

A calculation was performed to estimate the error due to radially diverging lines and to find the permissible limit of number of black and white lines of the star.

2. Mathematical Analysis

To simplify the calculation, a circular area with uniformly distributed intensity of light, which is a geometrical-optical approximation of defocussed point image, was adopted as the image of point.

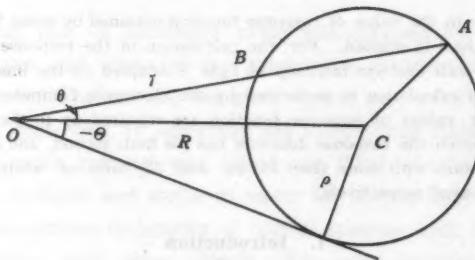


Fig. 1

Let us take a polar coordinate system (r, θ) , the origin of which is located in the centre of the star as shown in Fig. 1. Let a circle of radius ρ at the distance R from the origin O be the image of point. Assume A and B are intersections of a radius vector inclined to \overline{OC} at an angle of θ with the circumference of the circle C , $\overline{OB}=r_1$ and $\overline{OA}=r_2$, then the equation of the circle C is given,

$$\left. \begin{aligned} r_1 &= R \cos \theta - \sqrt{\rho^2 - R^2 \sin^2 \theta} \\ r_2 &= R \cos \theta + \sqrt{\rho^2 - R^2 \sin^2 \theta} \end{aligned} \right\} \quad (1)$$

The intensity of light is assumed to be unity inside the circle C and zero outside. Let the total number of pairs of black and white lines of the star be $2N$.

The value, obtained by scanning the point image with the star and measuring only the fundamental frequency component of the transmitted light, is equivalent to the value obtained by scanning with the chart the transmission of which varies as a function of angle θ as $\cos 2N\theta$ around the center of the star.

Therefore, excepting the constant factor, the value of the response function measured by the star can be determined by integrating $\cos 2N\theta$ inside the circle C .

Putting $R \sin \theta = \rho$, we obtain,

$$\begin{aligned} g(s) &= \int_{-\theta}^{+\theta} \int_{r_1}^{r_2} \cos 2N\theta \, r dr d\theta = \frac{1}{2} \int_{-\theta}^{+\theta} \cos 2N\theta (r_2^2 - r_1^2) d\theta \\ &= 2 \int_{-\theta}^{+\theta} R \cos \theta \sqrt{\rho^2 - R^2 \sin^2 \theta} \cos 2N\theta d\theta. \end{aligned} \quad (2)$$

In (2), the variable θ is changed to χ as $R \sin \theta = \rho \sin \chi$, giving

$$g(s) = 2\rho^2 \int_{-\pi/2}^{\pi/2} \cos^2 \chi \cos 2N\theta d\chi \quad (3)$$

Introducing the trigonometrical formula, i. e.,

$$\cos 2N\theta = 1 + \sum_{m=1}^N (-1)^m \frac{4N^2(4N^2-2^2) \dots [4N^2-(2m-2)^2]}{(2m)!} \sin^{2m}\theta \quad (4)$$

$$= 1 + \sum_{m=1}^N (-1)^m \frac{4N^2(4N^2-2^2) \dots [4N^2-(2m-2)^2]}{(2m)!} \frac{\rho^{2m}}{R^{2m}} \sin^{2m}\chi \quad (5)$$

into (3), we obtain

$$g(s) = 2\rho^2 \left[\int_{-\pi/2}^{\pi/2} \cos^2 \chi d\chi + \sum_{m=1}^N (-1)^m \frac{4N^2(4N^2-2^2) \dots [4N^2-(2m-2)^2]}{(2m)!} \frac{\rho^{2m}}{R^{2m}} \int_{-\pi/2}^{\pi/2} \cos^2 \chi \sin^{2m} \chi d\chi \right]. \quad (6)$$

To calculate (6), we need the following formula of definite integral,

$$\int_{-\pi/2}^{\pi/2} \cos^2 \chi \sin^{2m} \chi d\chi = \frac{1}{2m+2} \cdot \frac{2m-1}{2m} \cdot \frac{2m-3}{2m-2} \dots \frac{1}{2} \pi. \quad (7)$$

Substitute (7) into (6),

$$g(s) = 2\rho^2 \left[\frac{1}{2} \pi + \sum_{m=1}^N (-1)^m \frac{4N^2(4N^2-2^2) \dots [4N^2-(2m-2)^2]}{2^{2m+1}(m+1)! m!} \frac{\rho^{2m}}{R^{2m}} \pi \right] \quad (8)$$

where s is a measure of spatial frequency and $s = (2N)/R$. When s tends to zero by keeping N constant, R must increase indefinitely. Therefore,

$$g(0) = \pi \rho^2. \quad (9)$$

We obtain as the normalized response function $D(s)$,

$$D(s) = \frac{g(s)}{g(0)} = 1 + \sum_{m=1}^N (-1)^m \frac{4N^2(4N^2-2^2) \dots [4N^2-(2m-2)^2]}{2^{2m}(m+1)! m!} \frac{\rho^{2m}}{R^{2m}} \\ = 1 + \sum_{m=1}^N (-1)^m \frac{1}{(m+1)! m!} \left(1 - \frac{1}{N^2}\right) \left(1 - \frac{2^2}{N^2}\right) \dots \left(1 - \frac{(m-1)^2}{N^2}\right) \left(\frac{s\rho}{2}\right)^{2m} \quad (10)$$

If we assume $N \rightarrow \infty$ in (10), we have

$$D_\infty(s) = 1 + \sum_{m=1}^{\infty} (-1)^m \frac{1}{(m+1)! m!} \left(\frac{s\rho}{2}\right)^{2m} = \frac{2J_1(s\rho)}{s\rho}. \quad (11)$$

It means the Siemens star with infinite number of lines gives the correct value of response function for the image of point having uniform intensity distribution of light.

3. Numerical Calculation

Using the expression (10), the values of the response function measured by

Siemens stars of various numbers of lines, were calculated.

Two sets of values of N , 8, 16, 32, 64 and 12, 24, 48, 96 were adopted. $s\rho$ was changed between 0 and 10 at 0.2 intervals.

For the calculation, the electronic computer FUJIC made by the Fuji Photographic Film Co., was used. It took about 650 seconds to complete the calculation of 400 data.

Besides, for the cases of $N=36$ and $N=12$, calculations of 48 data in total were made by the automatic relay computer FACOM-128. The time needed for the calculations was approximately 55 minutes.

The results for $N=12$, obtained by both computers, were in complete accordance within the accuracy of calculation.

4. Results

The results obtained are shown in Fig. 2. In the figure, only the curves for $N=\infty$, $N=12$ and $N=8$ are plotted, because the curves for large values of N are scarcely different from the curve $2J_1(s\rho)/s\rho$ for $N=\infty$.

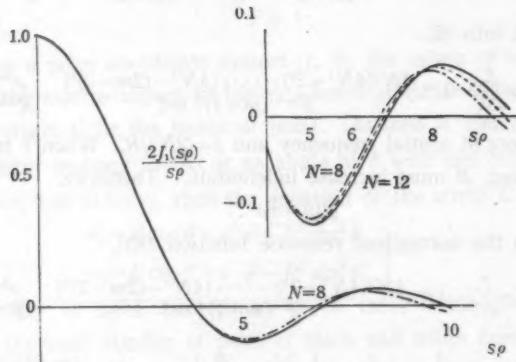


Fig. 2

The following conclusion can be deduced from this result. The difference between the values of response function obtained by scanning the point image with Siemens star and those obtained with the chart having black and white parallel lines is rather small and if correct values of response function are required up to the points at which the response function has the first, second, and third zeroes, the permissible lower limits of $2N$ should be 16, 24, and 32 respectively, that is, the Siemens star with 16, 24, and 32 pairs of black and white lines are tolerable in each case.

The smaller the number of lines, the wider the range of spatial frequency

covered by Siemens stars of the same size. Therefore the star with the number of lines as small as possible is preferable for measurement.

The above calculation was performed only for a special image of point having uniform intensity distribution of light. Hence, the results obtained are not applicable to other cases in every detail. Especially in the case of image of point having the asymmetry in radial direction of the star, more significant differences may appear. But the results obtained may throw some light on the feature of the problem.

The author wishes to express his thanks to Mr. Bunji Okazaki, Fuji Photographic Camera Co., for co-operation in performing the calculation with the electronic computer FUJIC.

Flame Spectrochemical Studies of Biochemical Materials.

I. Sampling and Preparatory Treating.

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(Received March 1, 1958)

Abstract

Biochemical materials are oxidized in liquid state, that is so-called wet-ashed, with the aid of iodine, 30% hydrogen peroxide and concentrated nitric acid, and after that, dissolved into the state of aqueous solution with hydrochloric acid. Iodine is a requisite substance to seize and oxidize viscous or fatty and oily materials, though it may be omitted for calcareous solids.

So-called dry-ashing method is found as inappropriate for the purpose of quantitative flame spectrochemical analysis especially on alcali and alkaline earth elements.

Photographically self-recorded flame spectral characteristic curves are highly useful in these studies due to their accurate reproduction of interfering effect caused by co-existing substances.

1. Introduction

Many studies on the determination of elements contained in biochemical materials have been reported in the past few decades.

These are chiefly purely chemical, and very few spectrochemical especially by flame spectral analysis. It is no exaggeration to say that it has only been reported after the well-known work of Lundegårdh¹⁾.

Since 1941 when the achievement of Beckman Photoelectric Spectrophotometer was reported in the Journ. Opt. Soc. Amer., efforts have been directed to the spectrochemical analysis using this kind of spectrophotometer. Though soon after that many studies have been made on varieties of samples using this apparatus, there are only a few on biochemical materials, but the determination of alcali and alkaline earth elements has been the most important theme. Especially, determination of ratios among the contents of sodium, potassium, calcium, magnesium and phosphorus is still the urgent requirement if one tries to study physiological or pathological phenomena. For this, the contents of these elements must be determined as correctly as possible, and the analysis of other elements becomes secondary

1) Lundegårdh, H., "Die quantitative Spektralanalyse der Elemente". I, II. Gustav Fischer, Jena, (1929-1934).

proposition excepting specially important elements such as iron, zinc, manganese, silicon, lead and etc. As the flame spectrochemical analysis is the most adequate technique for the determination of alcali and alcaline earth elements, the whole contents of these elements in the sample must, first of all, be seized and then analyzed.

So-called dry ashing method is not adequate for this purpose, and wet ashing that means oxidizing and dissolving of samples in liquid state seems suitable.

In this study, several biochemical materials were examined, and a new procedure of treatment is presented using a strong oxidizing reagent mixture especially suitable for this kind of materials.

2. Experimental

(1) Apparatus.

(a) Spectrophotometer.

A photoelectric spectrophotometer of Type EPU-2A using R.C.A. 1P-28 photomultiplier tube, offered by Hitachi, Ltd., and a Beckman Model DU spectrophotometer fitted with ordinary red and blue sensitive phototubes were used. For flame spectroscopy, a red sensitive phototube was also used for the former.

(b) Flame attachment.

Hitachi spectrophotometer is fitted with Type H-2 flame photometry attachment, and Beckman spectrophotometer with 9200 oxy-hydrogen flame attachment.

The Type H-2 flame attachment can be used either with oxy-hydrogen or oxy-acetylene flame, and oxy-hydrogen flame was used in this study, for the Beckman 9200 flame attachment is always used with oxy-hydrogen flame and it is necessary to compare results obtained by these two spectrophotometers.

(c) Self-recording apparatus.

Hitachi spectrophotometer is also fitted with Type S-2 recording apparatus which records photographically the flame spectral characteristic curves on a roll of sensitive paper.

This apparatus is schematically shown in Fig. 1. It records two curves in sensitivity ratio 10:1 simultaneously so that the curve for low concentration of element can be clearly traced.

(2) Wet-oxidation procedures.

At first, finely cut sample material is kneaded, and pulverized one is brayed with finely pulverized re-sublimed iodine in a small agate mortar. In this procedure oily and fatty, viscous or soft samples are absorbed into the powder of iodine colouring to violet or brown for the most part. Quantity of iodine ought to be three to five times of the weight of sample; if less than this, unattacked parts of the sample may be floating on the surface of oxidation reagent afterwards.

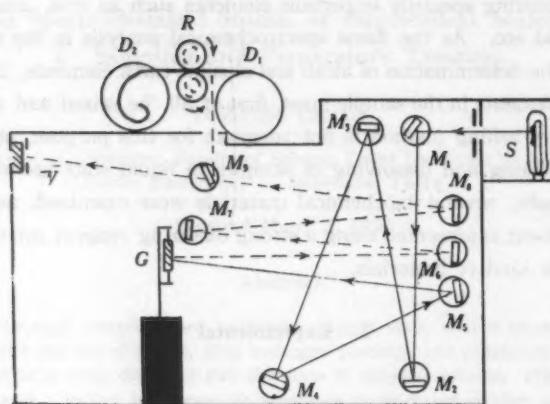


Fig. 1. Optical System of Type S-2 Recording Apparatus.
 G: Oscillating Mirror of Galvanometer.
 S: Light Source.
 M₁~M₆: Mirrors.
 D₁, D₂: Drums of Recording Paper.
 R: Rollers.

The mixture is then left over-night and thrown into concentrated nitric acid, not necessary fuming. After that, the whole mixture is heated slowly on a water-bath, and 30% hydrogen peroxide is added to it from time to time in two to three drops. This thick mixture is coloured violet or brown, but fades out little by little with the advance of oxidation. When it is wholly decolourized, no free iodine may be left, though considerable quantity of iodic acid (HIO_3) would remain in the solution. Iodic acid will react as a strongly oxidizing agent for the whole course of procedure.

Added reagents and iodic acid produced are all of considerably volatile nature, so the reaction vessel, say porcelain crucible or small evaporating dish, should be covered with a watch-glass of suitable size.

Achievement of oxidation is surmized by no oily substance floating on the surface of the solution, whole liquid being transparent with no oily, viscous or solid mass, and entirely decolourized when no iron exists in the sample.

If the sample contains iron, or is stained with blood, colour at the end point might be still slightly yellow by its Fe^{+++} -coloration. Remained iron can be certified by a wine-red coloration produced by the addition of potassium thiocyanate solution.

When this stage is reached, oxidation should be ceased, and total liquid is evaporated up slowly on a water-bath.

After no more of the white fume by evaporation of nitric acid is seen, a few drops of concentrated hydrochloric acid are added to the dried residue and again heated and dried up slowly on the water-bath. Although the residue could be changed into chloride in this operation, only one operation seems insufficient.

Repeating this operation two to three times, all metallic elements are presumed to have been changed into chloride; and lastly the residue is extracted with 1N-hydrochloric acid to make aliquot of solution reasonably fit for flame spectrochemical analysis. Occasionally with plant or vegetable materials, some white residue may remain undissolved. As it is silica for the most part, it may be separated by filtration to make the mother liquor to be of use for the flame analysis, and the residue should be analysed by some other suitable method.

(3) Purification of reagents.

For the purpose of purification of iodine, finely powdered "extra pure" class iodine and potassium iodide are mixed well in an agate mortar. Weight of the latter should be about twice of the former. This mixture is then transferred into a beaker and heated gradually on a sand bath. A flask into which cold tap water circulated is set up on the beaker to cool the sublimed iodine vapour and let it re-crystallize on the surface of the bottom of the flask. Repeating this procedure twice, iodine is obtained in a thoroughly purified state.

Other oxidizing agents, 30% hydrogen peroxide and concentrated nitric acid are re-distilled in reduced pressure. Most of the spectroscopically interfering substances in these liquid oxidizing agents are sodium, potassium and/or calcium originated from glass bottle, but these can be driven away almost perfectly if the re-distillation procedure is performed with adequate caution.

Hydrochloric acid is also purified with re-distillation under normal pressure. As the contamination of alcali or alcaline earth elements is originated for the most part from hydrochloric acid, re-distillation should be performed in a pyrex-like hard glass apparatus, and the distilled hydrochloric acid should be stored in a bottle of this sort of hard glass or of polyethylene.

(4) Pre-operation treatment of materials.

Sample materials must be cut or broken up into small grains as finely as possible. If it contains considerable water, some dehydration procedure is to be carried out before the oxidation.

Such dehydration is made most successfully either by preservation in a reduced pressure desiccator containing sulphuric acid or by treatment with acetone-ethanol mixture²⁾. In the latter case, some constituent might be extracted with the mixture;

2) Owen, L. E., Ellenburg, J. Y., *Appl. Spectroscopy*, **5**, No. 4, pp. 19 (1951).

it is preferable, therefore, to evaporate up the extract in reduced pressure and the residue is added to the sample in oxidation course. If dehydration is not carried out, the oxidation time is inevitably prolonged by the dilution of oxidation reagents with water, and some constituents may get lost by puffing of thick sample solution. Liquid biochemical samples such as serum, cerebrospinal liquor, urine or abdominal dropsy, can be thoroughly analysed after dehydration in a desiccator and subsequent oxidation.

3. Discussion on result

(1) The apparatus.

Use of photographic self-recording apparatus is advantageous for flame spectrochemical studies. Although indispensably slow in time required for developing, fixing, washing and drying, it is still superior to the pen-recording system because of its rapid and accurate response. Flame spectra contain several sharp atomic emission lines which are difficult to be recorded correctly with a pen-recording system unless the scanning speed is considerably low, however on the other hand, as the consumption of sample solution should be very little, the scanning has to be quickened as much as possible. These opposing requirements are reasonably satisfied in the photographing system as the motion of the galvanometer mirror is far swifter and more correct than that of recording pen, and Hitachi recording apparatus of Type S-2 is found appropriate for quantitative as well as qualitative or semi-quantitative analysis.

Its reproducibility is with 0.35 to 0.50% of relative deviation, when estimated with sodium 589 m μ line emitted from an aqueous solution containing sodium chloride in 0.0001 mole/l. concentration.

(2) The preparatory treatment of sample materials.

(a) Simple dilution method.

If merely the contents of sodium, potassium and calcium are dealt with, analysis may be performed directly with flame spectrophotometry simply by diluting the samples with water.

Simple dilution method is now being carried out widely but rashly in medical laboratories. Although it is considered as a very rapid and easy technique at a glance, it is yet doubtful as to correct results, for very strongly interfering substances, protein for example, will still be left in the diluted solution. To avoid this difficulty, the troublesome substance will have to be destroyed to let the sample solution grow to a simple inorganic salt solution, or else the interfering

effect removed or at least reduced from the results obtained as previously reported by the author et al³⁾.

(b) The customary ashing method.

Various procedures of dry and wet ashing methods have been reported. They are all very time-consuming and with errors in consequence. Of all biochemical materials, on the other hand, animal protein is most difficult to be oxidized and dissolved to form a homogeneous solution. On the contrary, vegetable protein is fairly easy to be dissolved.

Simply for destroying protein, dry method gives results more readily than wet method; however as the dry method is always based upon calcination of the material even if some oxidation reagent be added or calcined in lower temperature, alcali metal elements, especially potassium which is a very important element in biochemistry, tend to volatilize and may easily be lost. For example, in Fig. 2,

one can see the disadvantage of dry method.

In 1955, when the author co-operated with the members of Hitachi, Ltd., S. Shinoda, S. Sato and T. Kobayashi, some plant leaves were calcined to ash in an electric furnace at the temperature regulated not to exceed 400°C; the ash was then extracted with hydrochloric acid, and studied flame spectrochemically using a Type EPU-2A spectrophotometer and Type S-2 recording apparatus. Results were very interesting. There appeared fine characteristic curves and especially the 403.4 m μ peak of manganese line—correctly said, Mn I 4033.073 and Mn I 4034.490, excitation potentials of which are both 3.1 volts—were clearly resolved from the adjacent potassium line K I 404.4 m μ and correctly recorded (Fig. 2, (a) and (b)).

As it was thought some small quantity of alcali elements might have been lost, analysis was repeated with a similar apparatus on similar samples which were treated by wet method

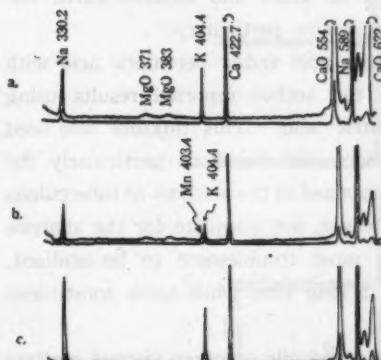


Fig. 2. Flame spectral characteristic curves of "dry" and "wet" ashed leaves.

- a: Dry-ashed leaf of peach.
- b: Dry-ashed leaf of chestnut-tree.
- c: Wet-ashed leaf of chestnut-tree.

I 404.4 m μ and correctly recorded (Fig. 2, (a) and (b)). As it was thought some small quantity of alcali elements might have been lost, analysis was repeated with a similar apparatus on similar samples which were treated by wet method using 30% hydrogen peroxide and concentrated nitric acid as the oxidation reagent. Result is shown in Fig. 2, (c), and there appear clearly high peaks characterizing alcali metal elements. These peaks are higher than those obtained in the

3) For example, Suzuki, M., Ohyagi, Y., Japan. J. Clin. Pathol., (Rinshō Byōri), 4, 245 (1956). (Printed in Japanese).

previous work (curves (a) and (b)), even if the heights of corresponding peaks by blank test are deducted from them. From these results, it is evident that alcalimetal elements may be lost even if the dry method is performed under gentle condition.

In a number of reported studies on wet ashing methods, various oxidation reagents were used such as fuming nitric acid, sulphuric and nitric acid mixture, aqua regia and sulphuric acid mixture, sulphuric and perchloric acid mixture and etc. Whichever reagent is used, errors due to contamination are far larger than those in the dry ashing methods; however, loss of alcali or alkaline earth elements is fairly small. Consequently, with due attention paid to the contamination, one can diminish errors in quantitative results.

On the other hand, sulphuric acid, though strongly oxidative and for that reason it is being used by many workers most profitably for destroying protein and other organic high polymers, is not recommendable in flame spectroscopy, for it seriously interferes with flame luminosity of alcali and alkaline earth elements, calcium, magnesium, strontium and barium in particular.

Some authors proposed the mixture of nitric acid and/or perchloric acid with hydrochloric acid for flame photometry; and the author reported results using 30% hydrogen peroxide and concentrated nitric acid. This mixture was used also by others and seems fairly convenient for solid materials particularly for calcareous ones, and good results have been obtained in the analysis of tuberculous calcified foci and of bone⁴⁾⁵⁾. It seems, however, not adequate for the analysis of liquid or viscous samples; oils and fats are most troublesome to be oxidized, floating on the surface and not reacted for a long time while some constituent might easily be lost.

A new procedure was devised to hold fats and oils or other viscous matters in the oxidizing reagent.

(c) The new procedure using iodine.

The agent that hold fats and oils or other viscous matters and does not let them float must be of large specific gravity, entirely free from any bio-element which would affect the flame and yet necessarily oxidizing. Among various substances examined, elemental iodine was found suited for the purpose. Iodine is a well-known bio-element and is surpassing in that, (1) it is not sensitive to emission spectra including those of flame excepting the I₂-bands (cf. Fig. 3), (2) its specific gravity is 4.93, (3) it can be purified easily by re-sublimation and (4) it is strongly oxidizing.

4) Ohyagi, Y., Tamura, S., Takahashi, M., J. Spectr. Soc. Japan (Bunkō Kenkyū), 5, No. 4, pp. 22 (1957). (In Japanese).

5) Ohyagi, Y., Takahashi, M., ibid., in printing.

At first, flame characteristic curve of iodine solution was examined. According to Gaydon⁶⁾, IO-bands will be emitted from flames of solutions containing iodine and methyl iodide. Besides, there may be chance for I₂-bands to appear. As iodine is strongly oxidizing, it should be consumed thoroughly in the course of oxidation of samples. Should the oxidation be interrupted half-way, there is a possibility of interference by the remaining iodine. Iodine may possibly be oxidized to iodic acid by the action of 30% hydrogen peroxide and concentrated nitric acid; the iodic

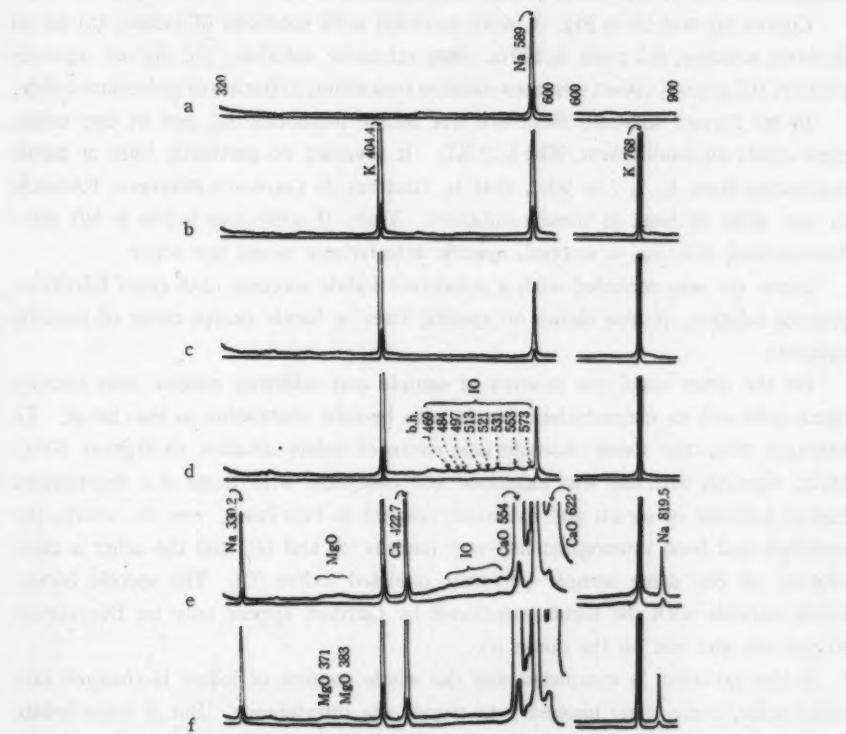


Fig. 3. Flame spectral characteristic curves concerning several states of iodine.

- a: Ethanolic iodine solution.
- b: Aqueous iodine solution with potassium iodide.
- c: Aqueous potassium iodide solution.
- d: Aqueous potassium iodate solution.
- e: Human serum oxidized to the halfway by the mixed oxidation reagent.
- f: Human serum oxidized and destroyed completely.

6) Pearse, R. W. B., Gaydon, A. G., "The Identification of Molecular Spectra". Chapman & Hall, London, (1950), pp. 150.

acid (or in the form of iodate) may further be reduced to iodide by the action of biochemical sample itself.

These possibilities were examined on the flame characteristic curves shown in Fig. 3.

Gaydon pointed out that IO-bands would appear most strongly in the region just above the inner cone of the flame⁶. For observation, therefore, the flame was carefully controlled by adjusting the pressure and flow volume to bring the part characteristic to IO-bands right onto the optical path.

Curves (a) and (b) in Fig. 3, were recorded with solutions of iodine; (a) by an alcoholic solution, 0.2 gram I_2 /100 cc.-98% ethanolic solution; (b) by an aqueous solution, 0.2 gram I_2 /100 cc.-aqueous solution containing 2.00 gram of potassium iodide.

In the former solution, there are free iodine molecules (I_2), and in the latter, there exists an equilibrium, $KI + I_2 \rightleftharpoons KI_3$. It revealed no particular lines or bands originating from I_2 , I_3^- , or IO_3^- , that is, contrary to Gaydon's inference, IO-bands do not arise at least in these conditions. Then, if some free iodine is left after the oxidizing reaction is stopped, specific interference would not occur.

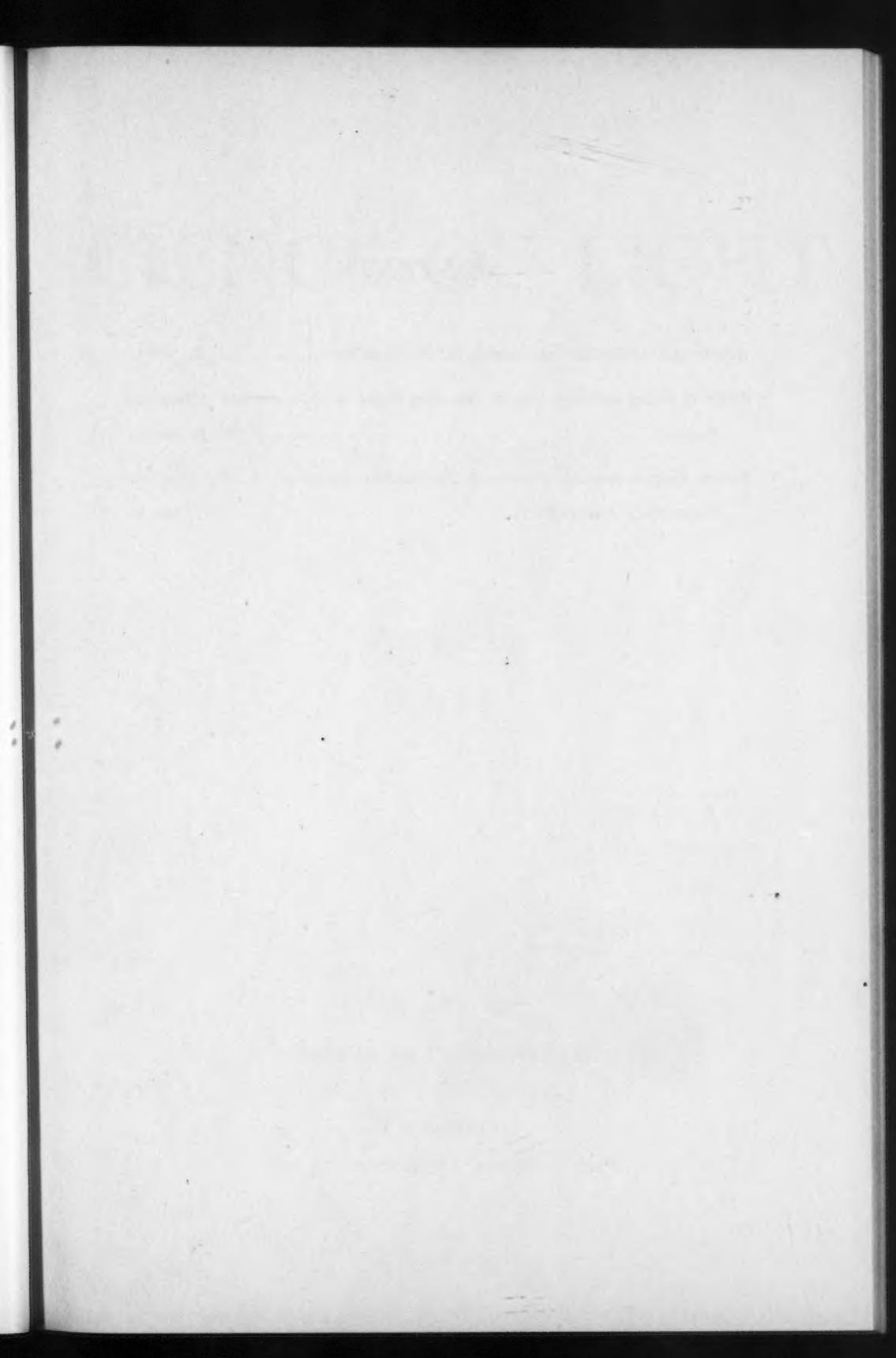
Curve (c) was recorded with a potassium iodide solution, 2.00 gram KI /100 cc. aqueous solution; it also shows no specific lines or bands except those of metallic elements.

On the other hand, the mixture of sample and oxidizing reagent may contain iodate ions and its characteristic bands may become observable in the flame. To ascertain this, the flame characteristic curve of iodate solution (0.20 gram KIO_3 /100 cc. aqueous solution) was examined and compared with those of a supernatant fluid of mixture of serum and oxidation reagent in two cases, one in which the oxidation had been interrupted half-way (curves (d) and (e)) and the other a clear solution of the same sample perfectly oxidized (curve (f)). The specific bands, which coincide with the bands mentioned by Gaydon, appear only on the curves (d) and (e), and not on the curve (f).

If the oxidation is completed and the whole content of iodine is changed into iodate state, there is no necessary to regard the interference. But if some iodate is left in the solution, interference may arise although slight. Therefore, oxidation procedure should be continued for some long time, say five to eight hours, till whole iodic acid is thoroughly consumed to ensure correct analytical results. The oxidation time required seems rather long, but it is far shorter than in any of other wet ashing methods.

Acknowledgments

The author wishes to express his hearty thanks to Miss Mieko Suzuki for her earnest assistance and co-operation.



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